Environment Protection Engineering

Vol. 47 2021 No. 4

DOI:10.37190/epe210405

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METHODS OF PROCESSING WHEY WASTE FROM DAIRIES. A REVIEW

The purpose of the study was to evaluate the methods of processing whey. The valorization method is suitable for the use of cheese whey and whey permeate to produce beverages with or without microbial conversion. However, this method does not ensure microbial conversion of lactose. Therefore, the organic load will not be reduced. The main advantage of aerobic decomposition is the relatively rapid degradation of organic matter. However, the high organic load in the crude cheese whey makes aerobic decomposition unsuitable and restrictions on oxygen transport may occur. Anaerobic decomposition can be used in various areas for the treatment of waste with a high organic load. The disadvantage of anaerobic processes is a higher cost compared to aerobic treatment. The combination of individual technologies significantly reduces the hydraulic retention time of the aerobic process and improves waste treatment. At present, there is a lack of studies in this area.

ABBREVIATIONS

AABs – acetic acid bacteria
AF – anaerobic filter reactor
ALG – alginate and chitosan polyion complex
AD – anaerobic digestion
AnMBR – anaerobic membrane bioreactor
AnSBR – anaerobic sequencing batch reactor
ASB – anaerobic sludge blanket
BDD – boron-enriched diamond
BOD – biological oxygen demand, mg/dm^3
CAR – chitosan-carrageenan complex
COD – chemical oxygen demand, mg/dm^3

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CW – cheese whey
EGSB – expanded granular sludge bed
FFR – fixed film reactor
HRT – hydraulic retention time, h
Chi-Pol – chitosan-polyanion complex
JLBRs – jet loop bioreactors
LAB – lactic acid bacteria
MBR – integrated membrane bioreactor
NRBC – nonwoven rotary biological contactor
OLR – organic loading rate, kg/(m^3·day)
sCOD – soluble chemical oxygen demand, mg/dm^3
SCW – secondary cheese whey
SCWO – supercritical water oxidation
$T(C)$ – total cycle time
TOC – total organic carbon,%
UASB – up-flow anaerobic sludge blanket
UASFF – up-flow anaerobic sludge fixed film reactor
UFFR – up-flow fixed-film reactor
VFAs – volatile fatty acids
VS – volatile solids
WP – whey permeate
WC – whey concentrate
WPC – whey protein concentrate
WPI – whey protein isolate

1. INTRODUCTION

The dairy industry is based on the processing and production of raw milk for end products – yogurt, ice cream, butter, cheese, and various types of desserts, using various processes. These processes include pasteurization, coagulation, filtration, centrifugation, cooling [1]. The dairy industry is divided into several sectors linked to the production of polluted water. The composition of this wastewater depends on the required final product [2]. One of the main sectors of the dairy industry is cheese production. The output of cheese production contains three main flows: cheese whey (CW – derived from cheese production), secondary cheese whey (SCW – derived from curd cheese production), and wastewater from pipes and other equipment [3]. Approximately 9 kg of whey is obtained from the production of 1 kg of cheese. Due to the low concentration of milk components (whey contains only 6–7 wt. % of dry matter), whey is commonly considered as waste. In terms of organic solids composition, CW contains mainly saccharides (4–5 wt. %, of which lactose has the highest concentration), proteins (0.6–0.8 wt. %), and lipids (0.4–0.5 wt. %). Whey is one of the most polluted waste flows from cheese production. Whey also contains milk proteins, water-soluble vitamins and mineral salts. In addition, waste whey is characterized by high levels of chemical oxygen demand

(COD) and biological oxygen demand (BOD), turbidity, oils and fats, suspended solids, phosphorus, and nitrogen [4].

Due to the above-mentioned composition, waste whey poses a significant risk to the environment when released directly into a watercourse. The high content of biodegradable organic substances and the flotation of fats causes rapid oxygen consumption and the start of anaerobic processes in aquatic ecosystems, which threatens the survival of aquatic organisms. The presence of lactose, nitrogen, and phosphorus can lead to the development and growth of fungi and algae with a consequent reduction in water quality. Acidic reaction and high salinity are indicators of contamination that must also be taken into account. Based on the above, it is necessary to consider appropriate ways of managing this waste flow [2]. All wastewater from milk production can be treated together. The exception is whey. Its complex biodegradability requirements can place too much burden on any wastewater treatment system. Therefore, waste whey should be treated separately from other dairy discharges [5].

Practically, it is possible to apply three basic options for processing waste flows from cheese production – valorization technology, physicochemical treatment, and biological treatment [5]. The purpose of valorization technology is to obtain valuable compounds such as protein and lactose. Each liter of cheese whey contains about 50 g of lactose and 10 g of high nutritional protein. The purpose of physicochemical processes (precipitation, membrane separation) is the production of whey powder, whey concentrate (WC), lactose, or minerals. In contrast, biological processes represent the microbial conversion of lactose, which is present in CW, SCW to organic acids, bioalcohols, methane, and hydrogen [6]. Physicochemical treatments are especially suitable for dairy companies with a large volume of processing and sufficient capital to invest in their implementation. On the contrary, for small and medium-sized enterprises, the removal of CW is a challenge because they do not have the economic resources needed for proper treatment and recovery. Therefore, these companies prefer to give residues to feed livestock or discharge them into the municipal sewer system, which can pose a serious threat to the environment [5].

Among mentioned options, biological treatment of waste whey is preferred. Biological processing offers a high degree of decomposition of organic compounds. In addition, physicochemical processes increase costs by using treatment agents. Although it should be added that these processes are effective in removing emulsified compounds [7]. Biological methods, aerobic or anaerobic, are used to remove organic matter from dairy waste. These treatments can be combined (see next chapter) to achieve wastewater discharge limits for dairy wastewater [8].

The purpose of the study is to describe the biological methods of processing whey waste, or their combinations. The study, according to the literature overview, initially analyses the composition of the waste flow from cheese production. The discussion focuses on the main advantages and disadvantages of biological methods of whey processing. At the end of the article, a proposal for future research is presented.

Generally, whey is divided into two basic types. The production of ripe cheese produces sweet whey (pH 5.8–6.6) and the production of curd cheese makes sour whey (pH 3.6–5.1) [9]. The main physicochemical properties are summarized in Table 1.

Table 1

Physicochemical parameters of cheese whey

pH	COD [g/dm^3]	BOD [g/dm^3]	Fat [g/dm^3]	TP [g/dm^3]	TKN [g/dm^3]	TSS [g/dm^3]	VSS [g/dm^3]	Reference
3.92	5.25±1.34			0.12	0.15	9.38±0.45	8.28±0.40	[10]
4.90±0.27	68.60±3.30	37.71±2.84	9.44±1.14	0.5	1.12	5.93±0.38a	5.61±0.36a	[11]
6.00–6.50	50.00–70.00	27.00–36.00			0.02–0.01	55.00–65.00		[12]
	18.5±1.4	14.8±1.5		0.007	0.338±0.02	7.650±0.6		[13]

2. VALORISATION OF RAW WHEY

Valorization technologies include physicochemical methods (i.e., protein precipitation and membrane separation) for the production of whey powder, whey protein concentrate, whey protein isolate, whey permeate, lactose and minerals, and biological methods. However, biological methods include the microbial conversion of lactose present in CW, SCW or raw whey permeate, to organic acids, bioalcohols, greenhouse gases (e.g., hydrogen, methane), and bioplastics [14–16]. Fermentation can significantly reduce the organic load (lactose content), thus enabling an economical and feasible alternative use of raw whey and at the same time reducing the environmental impact [16].

Hydrogen production with co-removal of COD was performed by electrohydrolysis of whey solutions with different initial concentrations of COD at a constant voltage of 3 V [17]. Electrons supplied by a direct current source reacted with hydrogen ions (H_2) released from the dissociation of VFAs produced by bacterial fermentation to form H_2 gas. The cumulative volume and rate of H_2 formation increased with increasing initial COD level. The highest cumulative volume of hydrogen (3,923 cm^3), the rate of hydrogen gas formation (699 cm^3/day), the hydrogen yield (1,719 cm^3/g COD) were obtained at the highest COD level of 25.025 mg O_2/dm^3 with 90.3% H_2 in the gas phase. The COD removal percentage ranged between 18 and 20%. The highest energy efficiency (93%) was obtained with an initial COD of 4 850 mg O_2/dm^3.

The use of CW and WP for the production of beverages, with or without microbial conversion, is one of the most attractive options for the valorization and use of whey for human consumption. Whey drinks are produced using simple technologies and are characterized by high nutritional value due to the presence of proteins and peptides with several biological and health-promoting functions (e.g., antioxidant, anti-inflammatory, anti-cancer, immunomodulatory, cardioprotective, and hypotensive activities) [18]. On the other hand, the high concentration of lactose causes these products to spoil rapidly.

There are several technological solutions to overcome these shortcomings, including pH adjustment, flavor supplementation, and microbial fermentation.

Fermentation is one of the cheapest ways to preserve food, improve nutritional value and sensory properties. CW or milk enriched with CW, WPC or WPI is suitable for the production of fermented beverages using yeasts and lactic acid bacteria (LAB). As with other fermented milk beverages, LABs can improve the shelf life, nutritional (e.g, protein degradation, production of bioactive peptides), and sensory properties (e.g, production of lactic acid and aromatic compounds) whey beverages [19]. Representatives of the genera *Lactobacillus* and *Streptococcus* are most often used for the preparation of whey drinks, although yogurt bacteria *L. delbrueckii* subsp. *bulgaricus* and *S. thermophiles* are used for the preparation of yogurts. However, the challenge in the whey beverage segment is certainly the use of probiotic strains. Species used mainly to produce functional whey beverages include *L. acidophilus*, *L. casei*, *L. rhamnosus*, and *L. reuteri* [20]. Bioconversion to fermented beverages would also allow the valorisation of raw whey in small and medium-sized cheese factories, which fail to meet the operating and production costs for the production of other whey products (e.g., whey protein isolates, whey protein concentrates, purified organic acids).

Bioconversion of whey and derivatives to alcoholic and vinegar beverages, including vinegar, is an interesting alternative to the production of dairy beverages for the production of new food commodities from dairy waste. The biological production of ethanol from whey requires microorganisms, mostly yeast, suitable for the assimilation of lactose to ethanol. The species *Kluyveromyces lactis* and *Kluyveromyces marxianus* (synonyms *Kluyveromyces fragilis* nom. Inval. and *Candida pseudotropicalis*) are lactose-fermenting yeasts. The maximum theoretical yield of ethanol from lactose is 0.538 g/g lactose; the fermented product thus contains approximately 3–5 wt. % of ethanol, depending on the technology and strain used [19]. The fermentation product is then centrifuged to remove the biomass and sent to a distillation column in which the ethanol content is increased to 95 wt. %. Unlike *Kluyveromyces* spp., the best alcohol-producing yeasts *Saccharomyces cerevisiae* are not able to ferment lactose and therefore cannot be used to produce ethanol from CW and other derivatives (SCW and whey permeate) without prior enzymatic hydrolysis of lactose to glucose and galactose [21, 22].

CW and derivatives after alcoholic fermentation can reach an ethanol content ca. 6 vol. %, which allows the production of vinegar and soft drinks. This way of valuing CW is in line with consumer demand for high-value products and government initiatives to promote healthy food and beverages. From a biotechnological point of view, the conversion of whey ethanol to acetic acid by acetic fermentation bacteria is feasible. Acetic acid bacteria (AABs) can produce acetic acid in fermenting fluids in which the ethanol content is in the range from 2–3 vol. % to 15–18 vol. %, depending on the fermentation system and the microbial strain used [23]. This wide range makes it possible to design versatile bioprocesses obtaining vinegar and beverages with a variable content of acetic acid and residual ethanol [19].

The conversion of CW to whey vinegar represents a valuable option for whey recycling in the chain of traditional fermented food while avoiding the main disadvantages of low productivity in the production of bioethanol from whey. The basic process is the bioconversion of sugars to ethanol using the lactose-fermenting yeast *Kluyveromyces*, which is further converted to acetic acid by AABs [24]. The best known is wine and apple cider vinegar, however, kinds of vinegar can be made from other unconventional sources containing sugars, such as CW and SCW, rich in lactose. Vinegar from CW and its derivatives is produced mainly in Switzerland. Because an amount of ethanol higher than 5–6 wt. % could inhibit the activity of AABs, *Kluyveromyces yeast* grown in whey permeate with lactose up to 200 g/dm^3 provide enough alcohol for the subsequent formation of acetic acid [19].

3. AEROBIC TREATMENT OF WASTE CHEESE WHEY

Aerobic treatment is the microbial decomposition associated with the oxidation of waste in the presence of oxygen. For conventional treatment of dairy waste by aerobic digestion, an activated sludge system, trickling filters, aerated lagoons, or a combination of these technologies can be used. Experiments were performed in suspended growth reactors (aerobic treatment) at different whey concentrations. Based on the study, the maximum level of organic load degradation was determined at a concentration of 100% waste cheese whey [25].

Aerobic decomposition is characterized by relatively rapid degradation of organic matter at room temperature (22–24 °C), which requires a short hydraulic retention time (HRT). However, the high organic load in the crude CW makes aerobic decomposition unsuitable. The optimal C/N/P ratio in aerobic processes is about 100/5/1 compared to 500/5/1 in anaerobic processes. Restrictions on oxygen transport may occur when handling highly polluted wastewater. In general, high contamination of raw milk effluents can cause excessive growth of fibrous microorganisms (volume increase) and consequent difficulties in sludge settling [26, 27]. As with anaerobic processes, proteins and fats can adversely affect sludge settling properties. Nonwoven rotary biological contactors (NRBCs) can withstand relatively strong discharges due to improved oxygen transfer. Therefore, in a study performed by Ebrahimi et al. [12] while using a three-step NRBC, they modified the crude CW with an initial COD of about 50 kg O_2/m^3. These authors reported COD removal in the range of 53–78% depending on HRT (8–16 h) with a residual COD 10.7–24.0 kg O_2/m^3. An anaerobic after-treatment was then applied for 16 h to finally reach the outflow, which represented the residual COD 1.6–2.6 kg/m^3. Consistently with previous claims, most previously published aerobic degradation studies have been performed with dilute CW. Therefore, the application of activated sludge to dilute CW was tested [26]. Two different dilution ratios and HRT were used (6 and 36 h), resulting in COD removal in the range of 93.6–95.3%. The residual COD achieved with the 1/100 CW dilution adjustment was below the legal limit value for direct discharge (150 mg O_2/dm^3). However, when the dilution was 1/10, the treated waste product showed a residual COD 1.73 times above the legal limit.

Among the advanced reactor configurations was the development of so-called jet loop bioreactors (JLBRs) – highly efficient compact reactors. These bioreactors are characterized by high oxygen transfer and mixing, turbulence capacity, small size, and reduced installation and energy consumption costs. Another efficient configuration is the membrane bioreactor (MBR) system [28]. The application of membrane units for solids separation can minimize the main disadvantages of conventional sedimentation at high biomass concentrations. Treated wastewater does not contain solids and infectious organisms.

Membrane JLBR reactors (JLMBRs) showed high COD reduction efficiency (99%) with residual COD below 5.8 kg O_2/m^3. These results were also obtained using high COD loads (range 3.5–33.5 kg O_2/m^3day). In addition, this technology can tolerate short time changes in the input organic load. When crude CW was used, COD removal decreased to 81–83%. JLMBR is also highly effective in removing total nitrogen (99%) and PO_4^{3-} ions (65–88%) [28]. Disadvantages include the fact that the generated sludge presents certain settling problems. Furthermore, the flow rate through the membranes decreases with the time of use.

4. ANAEROBIC TREATMENT OF WASTE WHEY

A number of pilot experiments have been carried out on the anaerobic treatment of waste whey in the past. However, it should be added that some of them worked with deproteinized or diluted whey, which is much easier to process [28].

Anaerobic decomposition is used in various areas for the treatment of waste with a high organic load. In the conversion of organic matter into biogas under anaerobic conditions, SO_2 and CO_2 ions are used as electron acceptors [28]. Anaerobic decomposition is usually carried out in two stages, in the first facultatively anaerobic bacteria convert complex organic compounds into simpler ones, e.g., volatile acids. In the second phase, strictly anaerobic bacteria convert the products formed by the first group into methane and carbon dioxide [7, 29]. Anaerobic decomposition in CW treatment reduces the discharge of pollutants, enables energy recovery and nutrient regeneration [30]. The application of anaerobic decomposition depends on:

- physicochemical composition of CW (organic matter, alkalinity, and tendency to rapid acidification),
- inoculum sources (high buffering capacity),
- reactor configurations (wastewater recirculation) [31].

Although raw whey has a high content of organic matter, the yield of methane is limited due to the production of volatile fatty acids during the fermentation of lactose.

Therefore, acid accumulation can lead to lowering of pH, growth of acetogenic bacteria, and inhibition of methanogenic activity. To maintain the optimal pH for methanogenic bacteria, it is appropriate to add substrates with buffering capacity to the whey [32]. During anaerobic decomposition, organic substances are stabilized and decomposed. However, during the process, most nutrients remain in the digestate in N/P ratios between 2 and 4 [33]. Although this digestate has good fertilizing properties, its direct application to crops has its disadvantages, such as ammonia emissions during irrigation [34] and the introduction of pathogens into fields. To solve these problems, practical solutions have been proposed for the extraction of nutrients from digestate. One of these alternatives is to obtain a fertilizer by precipitating ammonium magnesium phosphate hexahydrate ($NH_4MgPO_4 \cdot 6H_2O$), also known as struvite [35].

Struvite forms crystals by precipitation when the molar ratio of $Mg:NH_4:PO_4$ is above 1:1:1. Struvite has a lower solubility in water compared to commercial fertilizers, which improves its availability and inhibits the uncontrolled distribution of nutrients [36]. For the anaerobic digestate to precipitate with the struvite, the substrate must have two characteristics: availability and high nutrient content. Raw whey and its digestate have not been sufficiently researched to use this technology.

Inhibition by acidification is a common problem during the anaerobic degradation of acidic substrates such as whey. In a full-scale semi-continuous process, the use of whey as a substrate can lead to digester failure due to a lack of alkalinity and buffering capacity [31]. Based on a study performed by Escalante et al. [37], the use of stabilized cow manure as inoculum is proposed, which may help to achieve a balance between anaerobic digesters. This is because cow dung provides alkalinity (1850±175 mg/dm^3), other nutrients, and trace elements that are important for the growth of microorganisms. The advantages of using cow manure as an inoculum are its local availability, low cost, and ability to replace the chemicals needed to achieve a stable pH [38, 39].

The UASB reactor can cope with waste whey (pH ca. 4), even with increased OLR, which eliminates the need to replenish the alkalinity by ensuring proper commissioning. In addition to the anaerobic filter (AF), fixed-film reactor (FFR), and UASB reactors, hybrid and anaerobic sludge blanket (ASB) reactors are also used to treat dairy wastewater. The up-flow anaerobic sludge fixed film reactor (UASFF) reactor is a hybrid reactor that is a combination of the UASB and up-flow fixed-film reactor (UFFR) reactors and has been developed to shorten the commissioning time. This reactor was used for the rapid biological conversion of CW organics to biogas. At HRT 48 h and 36 °C, a COD removal rate of 97.5% was observed with a short start-up time. The highest biogas production rate 3.75 dm^3/day occurred at HRT of 36 h [40].

A new multilayer reactor for CW wastewater was tested at a cheese factory in Canada. The input COD ranged between 20 and 37 kg O_2/m^3 and OLR between (expressed as COD) 9 and 15 kg O_2/m^3. The maximum removal of COD was quite high – 92%. The level of biomass activity was maintained or increased during the research. The innovative reactor design seemed promising for CW processing and worked efficiently for one year. Some studies have developed anaerobic digestion processes for CW using cow dung. For example, in a study performed by Saddoud et al. [11], CW was decomposed in a membrane reactor to give a methane yield of 0.3 m^3/kg COD and varying organic load level (OLR) from 3 to 19.78 kg O_2/m^3day. In research described by Comino et al. [41], a stirred reactor was used to decompose CW mixed with a cow manure suspension at an OLR of 2.65 kg VS/m^3day to give a methane yield of 0.34 m^3/kg VS.

Similarly, in a study performed by Fernández et al. [42], a yield 0.314 m^3 CH_4/kg COD was demonstrated during two-stage anaerobic digestion (AD) of CW and sewage sludge under mesophilic conditions, with OLR 1.5 kg O_2/m^3day. In this case, the additional energy requirements create costs that are unsatisfactory for small and medium-sized enterprises. Therefore, the use of inexpensive tubular digesters is an interesting alternative for CW processing. In this type of reactor, acidogenic and methanogenic phases are separated, which improves the stability of the process [31]. In the study performed by Escalante et al. [31] of anaerobic decomposition of whey in a tubular reactor has been carried out. Whey as an acidic substrate tends to inhibit anaerobic degradation due to the accumulation of volatile fatty acids (5000 mg/dm^3) in the system. The combination of recirculation and the use of an auxiliary substrate improve the stability of the process. The anaerobic decomposition of whey mixed with fresh manure ensured pH stability in the range of 7.5–7.9 and the ratio of volatile fatty acids to total alkalinity was less than 0.4. Biogas production of biogas was achieved 0.409 m^3/kg VS with OLR 2 kg O_2/m^3day.

The advantage of anaerobic decomposition over aerobic is mainly in saving the cost of installing an oxygen injection system. Anaerobic treatment of whey produces a considerable amount of energy in the form of methane. In addition to the energy obtained by anaerobic decomposition, a significant amount of whey contamination is removed and nutrients are recovered in the form of digestate [37]. Anaerobic decomposition of waste whey can endanger the biomass in the fermenter. Since whey is rich in lactose, it tends to be rapidly acidified, leading to a decrease in pH to 4. At this pH, the concentration of undissociated VFAs is too high, causing inhibition of methanogens as well as destabilization of the fermenter itself.

In addition, not all cases of stand-alone anaerobic digestion are sufficiently effective in degrading COD from whey. Rapid acidification during anaerobic decomposition can result in sludge granulation. In addition, it is important to note that these systems are costly and require qualified staff [43]. The possibility of eliminating operational complications with low pH values is co-digestion. Mono-digestion of whey led to the accumulation of intermediates inhibiting the anaerobic degradation process. Co-digestion of whey with manure can alleviate severe acidification during decomposition [44]. Co-digestion was also used to mix sewage sludge with a dried mixture of food waste, raw whey, and wastewater in the production of olive oil. The added mixture at the addition of 3–5 vol.% caused an increase in methane production during anaerobic decomposition [45].

In addition to co-digestion, a suitable type of reactor can be used to stabilize the conditions of anaerobic decomposition of waste whey fermenters or a suitable type of anaerobic decomposition technology. Various types of reactors have been used to ensure the stability of the anaerobic decomposition of waste whey. Among these reactors, an anaerobic sequencing batch reactor (AnSBR) has proven to be suitable. Its advantages lie in flexibility to operating conditions such as mixing, the substrate to biomass ratio, feeding strategies. This type of reactor allows close contact between the biodegradable components of the substrate and bacteria, which provides advantages such as high biomass concentration and biogas production. In addition, it is highly effective in removing COD from heavily polluted wastewater [46]. A study performed by Fernández et al. [42] evaluated the thermophilic anaerobic degradation of waste whey using one-stage and two-stage digestion. According to this study, the anaerobic decomposition of raw whey under thermophilic conditions was successful at an HRT of 8.3 days with the methane yield being higher in the two-stage anaerobic decomposition compared to the single-stage.

Anaerobic membrane bioreactors can solve the problem of poor granulation and leaching of biomass while providing high and stable wastewater treatment efficiency. In general, a high COD removal efficiency of over 90% was observed in AnMBR. The reactor quickly regained its stability (>98% COD removal efficiency) as the volumetric loading rate decreased while maintaining a reduced nitrogen concentration [46]. Dereli et al. [46] state that nitrogen is a limiting factor that causes reactor instability in the accumulation of volatile fatty acids VFAs, especially propionic acid, and that a low COD to nitrogen ratio is required for efficient treatment of rapidly degradable wastewater. The specific methane formation in the reactor varied during the study between 0.24 and 0.30 Nm^3/kg COD. Total COD concentration in the effluent at an average OLR of 5 kg O_2/m^3day was 365 mg O_2/dm^3. It was even possible to achieve an average COD concentration in the wastewater of 55 mg O_2/dm^3 when the operating OLR was reduced to 2 kg O_2/m^3day [46].

Cruz-Salomón [47] tested the anaerobic decomposition of wastewater using the expanded granular sludge bed (EGSB) bioreactor as a possible new sustainable alternative for the treatment of this wastewater with bioenergy production. In this study, the bioreactor was operated under stable conditions (i.e., buffer index 0.23±0.1, pH 7.22±0.4, and temperature 26.6±1.4 °C) for 201 days. During the evaluation, the hydraulic retention time (HRT) was 6 and 8 days and was buffered with $NaHCO_3$. Under these conditions, the COD removal rate and biochemical methane potential (BMP) were 90, 92% and 334, 328 cm^3 CH_4/g COD, respectively. The results confirmed that cheese-making effluents can be efficiently treated in an EGSB bioreactor using buffer [47].

5. COMBINATIONS OF DIFFERENT METHODS OF WHEY PROCESSING

Operational complications can arise when biological processes for whey processing are applied alone. Changes in flow, composition as well as high lactose content of waste whey prevent the use of aerobic processes in small and medium-sized enterprises for cheese production. When anaerobic processes are applied alone, sludge flotation occurs due to the presence of fat and the potential leaching of active microbial biomass. In addition, casein requires specific microorganisms for its degradation. This implies the need for a combination of individual methods of processing whey. An example is a combination of coagulation, flocculation, and aerobic biological treatment. This combination could significantly reduce the hydraulic retention time of the aerobic process [48]. Another example is the combination of aerobic and anaerobic biodegradation for the complete treatment of waste whey. The first step is the anaerobic degradation of the main organic matter fraction. Anaerobic degradation is followed by aerobic treatment to reduce the final organic load of the wastewater to meet discharge requirements.

In the study performed by Prazeres et al. [49], wastewater was treated with a Fenton--like oxidation system after pretreatment through a step of coagulation-flocculation of $FeCl_3$ or a sedimentation step with $Ca(OH)_2$ followed by aerobic digestion. In the first case, Fenton-like oxidation can reduce the initial COD to 80% of the original value, 20% of the COD showing re-sensitivity to chemical oxidation regardless of the operating conditions used. In the latter case, the oxidation system can remove almost all of the COD present in the pretreated wastewater. Due to the lower values of the initial COD, complete conversion of the COD is achieved in short reaction times within a few min depending on the initial concentration of the reagent. Removal of Fe(III) from the oxidation treatment can be achieved by the addition of $Ca(OH)_2$. Sedimentation pH significantly affects the observed settling rate. Thus, neutral conditions lead to better results than slightly acidic pH.

Another method is acid precipitation, in which the pH is reduced to a range of 1–3 by the addition of sulfuric acid, leading to the formation of agglomerated particles and later a white precipitate. In the pH range 1–3, while turbidity is reduced by 12%, and nutrient (P and N) is removed at a level of about 18%. pH 2.0 allows significant removal of TSS (70.5%) but the supernatant shows a low value of biodegradability. As a result, this pH is not suitable as a pretreatment for biological processes [50].

Alkaline agents affect the diminishing of organic load and the biodegradability of the supernatant. The amount of sludge is also affected by the alkaline agent and is maximal with the maximum diminishing of the organic load with $FeSO_4$ and lime. On the other hand, at higher pH, the proteins contained in the wastewater are negatively charged in favor of coagulation. The removal of organic matter was higher using $Ca(OH)_2$. The use of $Al_2(SO_4)_3$ resulted in COD and BOD_5 reductions by 13 and 21%, respectively, when the alkaline reagent was NaOH and 35 and 36%, respectively, when $Ca(OH)_2$ was added [51].

Another example of a combination of biological methods is the combination of anaerobic digestion with an activated sludge aerobic system. Sequential anaerobic/aerobic wastewater treatment is gaining attention due to efficient nutrient removal, improved xenobiotic degradation, and removal of high BOD substances.

Complete treatment of whey effluent usually requires two steps, anaerobic degradation of the main organic matter fraction, then aerobic treatment of the partially treated effluent to reduce the final organic content of the effluent to meet discharge requirements. The aerobic step can be performed in aeration tanks [51]. The small industry cannot afford these systems and is therefore looking at alternatives to anaerobic fume hoods. The use of one fermenter for the main anaerobic treatment and the subsequent aerobic treatment could correspond to their financial possibilities. The gradual combination of anaerobic and aerobic degradation in a single digester has been designed as a means for small cheese producers to treat wastewater economically.

Sequential anaerobic and aerobic treatment of dairy wastewater has already been published with an SBR reactor treating wastewater from raw whey after previous anaerobic digestion in a hybrid reactor with inflow and outflow. SBR was used in anoxic and aerobic cycles at 24 h doses and achieved a COD removal of 88–94%. The main goal was to reduce the high concentration of nitrogen and phosphorus, which is still present in anaerobically treated whey wastewater. The different ratios of nutrients and biomass caused the removal of nitrogen up to 66–93% and the removal of 35–93% of phosphorus [52].

Most studies dealing with the sequential treatment of whey wastewater have been performed under mesophilic (35 °C) or thermophilic (55 °C) conditions, although psychrophilic digestion may lead to lower cleaning costs and become more suitable for small cheese producers. There is a need to further optimize and develop psychrophilic digestion to increase the applicability of the process and maximize the understanding of the microbiology of the process [52]. Preliminary studies of anaerobic and aerobic sequential treatment of whey effluent at psychrophilic temperature in a single fermentor have been demonstrated in 0.5 dm^3 SBR. SBR was run for 48 h cycles, with various levels of aeration after an initial anaerobic incubation of 30 h. The addition of 54 mg O_2/g COD over 16 h showed the best performance with sCOD removal reaching 99% and residual sCOD 104±22 mg O_2/dm^{-3}.

According to a study described by Frigon et al. [53], a total time of at least 3 days is required to achieve satisfactory removal of COD (97%) and residual sCOD (33 mg O_2/dm^3). The increase in aeration during the second phase – aerobic step did not improve the overall performance of the SBR with a residual sCOD of 463±122 mg O_2/dm^3. Methanogenic activity was low during most of the experiment, while acid-forming activity increased significantly over time. The concept of combining anaerobic and aerobic steps within a single fermenter still looks promising. Sequential anaerobic and aerobic degradation of whey effluent could be improved by dividing the anaerobic and aerobic biomass in the fermenter.

The effect of pretreatment of waste whey by precipitation was used in the study evaluated by Rivas et al. [13]. NaOH or $CaOH_2$ were used as precipitants. After pretreatment, the whey was treated by aerobic biodegradation. Both precipitants caused a 50% reduction in COD in the raw whey. When comparing the two precipitates, the resulting solid precipitate separated from the liquid mixture more easily after the addition of $CaOH_2$. In addition, sludge formation after the biological process is reduced to a minimum. The formed sludge shows good settling properties especially with the use of $CaOH_2$.

Tirado et al. [54] investigated in laboratory conditions the use of electrochemical methods consisting of electrocoagulation and electrochemical oxidation in the processing of waste whey. During electrocoagulation, electrodes made of various materials – Fe, Al, and stainless steel – were tested. Active and inactive anodes were used in the electrochemical oxidation. The optimal anode/cathode combination for electrocoagulation was Fe/AISI 304, which resulted in the highest removal of total organic carbon (TOC) of 22.0–27.0%. This is due to different effects on organic compounds: coagulation promoted by $Fe(OH)_3$ flocks, cathodic reduction, and oxidation generated by active chlorine. The highest TOC removal was achieved using boron-enriched diamond (BDD) anode due to the high oxidizing power of hydroxyl radicals. In contrast, total nitrogen was reduced much more rapidly with active anodes due to the attack of active chlorine on N-compounds.

Un et al. [55] performed whey wastewater treatment using a uniquely designed continuous electrocoagulation reactor. An empirical model of effective operating factors such as actual density, pH, and retention time was developed using the response surface methodology. The screw-type continuous electrocoagulation reactor was found to be effective in treating raw whey effluent. The initial concentration of COD 15 500 mg O_2/dm^3 was reduced to 2.112 mg O_2/dm^3 with a removal efficiency of 86.4%. Current density and retention time have a linear effect on the COD removal efficiency, while the initial pH of the wastewater has a quadratic effect on the COD removal efficiency concentrations reached a minimum value when the current density was 60 mA/cm^2, the retention time was 20 min and the initial pH was 4.54.

Electrochemical purification of deproteinated whey wastewater was performed using iron electrodes in the presence of NaCl electrolyte. This removal value is relatively higher than many other electrochemical processing processes, and the electrolysis time of 8 h is the main advantage of this method compared to conventional biological treatments. The electrochemical treatment conditions were optimized through response surface methodology (RSM), where the applied voltage was kept in the range, electrolyte concentration was minimized, waste concentration and COD removal percent were maximized at 25 °C [56].

Chitosan-polyanion (Chi-Pol) complexes have been used as coagulants to treat cheddar whey. Complexation and coagulation times played an important role in adsorption, while the concentration of polymers was significant only for chitosan-alginate complexes. Complexes of chitosan with alginate, pectin, and carrageenan used in a mixture of 30 mg/dm^3 whey showed a reduction in turbidity of 40–43% and 65–72% after 1 and 39 h. At 10 mg/dm^3, the percentage reduction in turbidity at 1 and 39 h was 35 to 39% and 61 to 64%, respectively. This study has successfully demonstrated the efficacy of Chi-Pol complexes in flocculating suspended solid waste in raw whey with more than 70% protein recovery [57].

The oxidative and hydrothermal decomposition of raw whey was investigated using a continuous flow reactor in supercritical water. The reaction conditions ranged between 400–650 °C and residence times of 6–21 s at a pressure of 25 MPa. The treatment efficacy based on TOC removal was achieved between 75.0% and 99.81%. The efficiency of the treatment increases with increasing temperature and the presence of excess oxygen in the reaction medium. Although the main gaseous products consisted of CO_2 and CH_4, gaseous products such as C_2H_6, C_3H_8, C_3H_6, CO, H_2, and N_2 exist in the waste phase in the gas phase and their amounts are negligible. The liquid phase is also clear and colorless. Therefore, the supercritical water oxidation process appears to be an effective technology for treating raw whey wastewater. This method may be suitable for reducing the contaminant load of raw whey water if energy is generated or energy consumption is reduced [58].

6. CONCLUSION

Anaerobic treatment of waste whey is the most effective way to remove organic pollution. The combination of the anaerobic method with the aerobic precursor further increases the degree of decomposition of the organic load. Compared to other methods, anaerobic treatment is the most researched area so far. Due to the evaluation of waste streams, the method of valorization of waste whey seems to be attractive. As follows from the literature, the valorization itself is based on the application of physicochemical processes. Compared to biological methods, valorization provides higher process stability, as at low pH whey, slowing down, even complete collapse of the whole process can occur in an anaerobic reactor. The application of the aerobic method alone is not sufficient in the decomposition of the organic portion of waste whey. Therefore, its application is suitable mainly in combination with subsequent anaerobic treatment. Valorization technologies should be used more for dairy companies with higher volume and capital due to the high cost of these technologies. As a space for further research, it is necessary to look for ways to minimize the costs of valorization technologies, which will make them attractive even for smaller dairies. The possibility of combining valorization technology with subsequent anaerobic degradation of the residual organic fraction is also interesting. At present, there is also a lack of studies aimed at comparing the costs of valorization technologies and the possible profit from the sale of individual products. Without economic efficiency, it is not possible to use any valorization technology even while ensuring the stability of processes and complete processing of waste whey.

ACKNOWLEDGMENTS

This research was funded by the research contract KEGA 008TU Z-4/2019. The paper is based on work performed under the research contract KEGA 008TU Z-4/2019 whose support is gratefully acknowledged.

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